1

DESCRIPTION

COMPOSITION FOR MEDICAL USE HAVING IMPROVED WATER-SOLUBILITY OF PEPTIDE AND METAL-LABELING EFFICIENCY AND PREPARATION FOR MEDICAL USE COMPRISING METAL-LABELED PEPTIDE

TECHNICAL FIELD

[0001]

The present invention relates to a medical composition having improved water-solubility and metal-labeling efficiency of a peptide capable of being labeled with a metal, which comprises a peptide capable of being labeled with a metal, to a medical preparation comprising the above-described peptide that is labeled with a metal, and to a method for labeling the above-described peptide with a metal. To be more specific, the present invention relates to a medical composition obtained by dissolving a peptide capable of being labeled with a metal, which is insoluble or poorly soluble in an aqueous solvent, together with a basic organic compound in the aqueous solvent, wherein the metal-labeling efficiency of the above-described peptide at room temperature improves; to a medical preparation obtained by labeling, with a metal, the above-described peptide in the above-described medical

composition; and to a method for labeling the above-described peptide with a metal.

BACKGROUND ART

[0002]

A peptide having a group, in the molecule, which is capable of being labeled with a metal can be used as an active ingredient in diagnostic or therapeutic agents by labeling the peptide with a radioactive metal, a paramagnetic metal, or the like.

The solubility of a peptide capable of being labeled with a metal decreases in an aqueous solvent with the increase in the number of hydrophobic moieties such as an alkyl group in the peptide structure. A conventional approach to dissolve such a peptide in the aqueous solvent is as follows: the peptide is dissolved in advance in an organic solvent such as dimethylsulfoxide (DMSO), dimethylformamide (DMF); and then the resulting solution is mixed with a required amount of water or a buffer solution or an aqueous solution of a certain substance. For example, formyl-Met-Leu-Phe (FMLP), one of peptides, is known to be poorly soluble in a neutral buffer solution. To dissolve the compound formyl-Met-Leu-Phe-Lys-hydrazinonicotinamide (fMLFK-HYNIC), which is a derivative of the compound FMLP to make it possible to be labeled with a metal, in an aqueous solvent, fMLFK-HYNIC is required to be dissolved with DMSO, followed

by adding a necessary buffer solution or aqueous solvent (see Non-Patent Document 1).

However, in the dissolution by the above-described method, upon mixing with the aqueous solvent, the proportion of the organic solvent, which is capable of dissolving the compound, decreases in the solution. When the organic solvent falls short of a certain concentration, sometimes the peptide may become insoluble such as white turbidity and deposition.

[0003]

As described above, the peptide capable of being labeled with a metal can be used as a nuclear medicine preparation by labeling it with a radioactive metal. Previous applications of dissolution of the above-described peptide with an organic solvent such as DMSO have required reaction conditions of heating at 100°C for more than 10 minutes or reaction conditions at room temperature for more than 30 minutes for the labeling.

For example, the labeling of the fMLFK-HYNIC requires reaction conditions at room temperature for a period of 30 to 60 minutes (see Non-Patent Document 1).

Peptide derivatives of FMLP are said to be useful in the imaging of disease with leukocyte infiltration such as inflammation, while conditions under which they are capable of being labeled with a metal at room temperature in a completely aqueous solvent are unknown (see Non-Patent Document 2).

[0004] .

Non-Patent Document 1: van der Laken, CJ. et al., J. Nucl. Med., 38, 8, 1310-1315 (1997)

Non-Patent Document 2: Verbeke, K. et al., Nuclear

5 Medicine & Biology, Vol. 27, 769-779 (2000)

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0005]

10   In view of the above-described problems, an object of the present invention is to provide a medical composition comprising a peptide, which is capable of being labeled with a metal without heating, wherein the peptide, which is insoluble or poorly soluble in water,

15 becomes easy to dissolve in an aqueous solvent; a medical preparation comprising the above-described peptide that is labeled with a metal; and a metal-labeling method for the above-described peptide.

20 MEASURE FOR SOLVING THE PROBLEM

[0006]

The present inventors have conducted diligent studies for attaining the object and have consequently completed the present invention by finding that, to our

25 surprise in terms of previous findings, the above-described peptide can be prepared easily into an aqueous solution at room temperature without the use of organic solvents and surfactants and becomes capable of

being labeled with a metal in a nonheated manner, by

adding the peptide to a basic organic compound.

[0007]

Specifically, the present invention relates

5   to a medical composition comprising a peptide capable

of being labeled with a metal and a basic organic

compound acceptable as a pharmaceutical additive.

The present invention further relates to a

freeze-dried medical composition characterized in that

10   the composition is obtained by freeze-drying the above-

described medical composition.

The present invention further relates to a

medical preparation characterized in that the

preparation is obtained by labeling, with a metal, a

15   peptide capable of being labeled with a metal in the

above-described medical composition.

The present invention further relates to a

method for labeling, with a metal, a peptide capable of

being labeled with a metal, characterized by comprising

20   dissolving the above-described peptide in an aqueous

solvent of a basic organic compound and then labeling

the resulting product with a metal.

The present invention further relates to a

method for producing a medical preparation comprising a

25   metal-labeled peptide, characterized by using the

above-described metal-labeling method.

EFFECT OF THE INVENTION

[0008].

The use of the medical composition according to the present invention has improved the solubility of a peptide capable of being labeled with a metal, which

5   is insoluble or poorly soluble in an aqueous solvent and allowed for the metal-labeling of the above-described peptide without heating. The use of the metal-labeling method according to the present invention has allowed for the labeling of the above-

10   described peptide with a metal under nonheated conditions. Furthermore, the preparation obtained by labeling, with a metal, the above-described peptide in the medical composition according to the present invention had the advantage of improving the

15   accumulation rate of the peptide to inflammation as compared with that of a composition prepared by a conventional method, for example when the peptide that can be used in the imaging of inflammation has been used therein. For example when a leukocyte-binding

20   compound has been used as the peptide, the present invention has been capable of providing a medical composition and a medical preparation and a labeling method thereof being useful in PET diagnostic imaging, SPECT diagnostic imaging, and MRI diagnostic imaging

25   that conduct the imaging of active leukocyte infiltration sites with the immune responses of individuals, or in radiation therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009]

Fig. 1 is a diagram showing the elution rates of Peptide 1, Peptide 2, and Peptide 3;

5 Fig. 2 is a diagram showing the comparison of the elution rates of Peptide 1 by the addition of a variety of additives at pH 9;

Fig. 3 is a diagram showing the image of a rabbit model of infectious disease injected of Peptide 10 1 labeled with Tc99m in a heated manner in dimethylformamide (DMF);

Fig. 4 is a diagram showing the image of a rabbit model of infectious disease injected of Peptide 1 labeled with Tc99m in a nonheated manner by use of 15 arginine; and

Fig. 5 is a diagram showing the accumulation (% ID/K pixel) of Peptides 1 labeled with Tc99m by different labeling methods to inflammation in a rabbit model of infectious disease.

20

BEST MODE FOR CARRYING OUT THE INVENTION

[0010]

Hereinafter, embodiments of the present invention will be described. All amino acids used 25 herein are indicated by one-character or three-character codes, and the N termini and C termini thereof are indicated in the left and right, respectively, unless otherwise stated. Peptides and

organic compounds bound with side chains are indicated
in parentheses subsequent to amino acids, unless
otherwise stated. The N termini and C termini of amino
acid sequences in parentheses are indicated in the

5   right and left, respectively, to easily understand the
whole structures. In the present specification, D-form
amino acids are referred to as D-amino acids.

[0011]

A medical composition according to the

10 present invention is specifically prepared by using a
peptide capable of being labeled with a metal, which is
insoluble or poorly soluble in an aqueous solvent, and
a basic organic compound acceptable as a pharmaceutical
additive as essential components to dissolve them in an

15 aqueous solvent.

Examples of the basic organic compound
include a basic amino acid and a basic compound having
an imidazole ring. Examples of the basic amino acid
include, but not particularly limited to, arginine,

20 lysine, histidine, and hydroxylysine. Preferably,
arginine, lysine, or histidine is used, and more
preferably, arginine is used. The basic compound
having an imidazole ring is not particularly limited
and can be exemplified by imidazole. While one type of

25 basic amino acid or basic compound having an imidazole
ring can be used alone, two types thereof may be used
in combination.

[0012]

When the basic organic compound is dissolved in an aqueous solvent together with the peptide capable of being labeled with a metal, it is preferred that the basic organic compound in the medical composition

5   should be contained in the concentration range of 0.1 mM to 1 M in the composition. The concentrations less than 0.1 mM are not preferable because labeling-promoting effect is not obtained. The concentrations higher than 1 M are not preferable because osmotic

10 pressure and toxicity become problems. The amount of the basic organic compound used differs depending on the type of the peptide used, and so on and is usually preferably in the molarity range of 10 to 1000000 times, more preferably 1000 to 100000 times, higher

15 than that of the peptide capable of being labeled with a metal. The molarities 10 or lower times higher than that of the peptide capable of being labeled with a metal are not preferable because sufficient dissolution-promoting effect and labeling-promoting

20 effect by the basic organic compound are not obtained. The molarities 1000000 or higher times higher than that of the peptide capable of being labeled with a metal are not preferable because the above-described basic organic compound existing in an excess amount has

25 inhibitory action on labeling.

In the above-described concentration range, the pH of a solution preferable as the composition ranges from 8 to 12, more preferably 8.5 to 12. The pH

more acidic than 8 is not preferable because
dissolution-promoting effect by the basic organic
compound is impaired. The pH more alkaline than 12 is
not preferable because it might adversely affect living

5 bodies.

[0013]

The peptide capable of being labeled with a
metal that is used in the present invention is
specifically a peptide insoluble or poorly soluble in

10 an aqueous solvent and is preferably a peptide
available as an active ingredient in a diagnostic drug
or a pharmaceutical drug for therapeutic use. A
polyamino acid compound having 30 or less amino acid
residues or a molecular weight of 4500 or less is more

15 preferable. The peptide may contain an amino group or
carbonyl group in its structure. The above-described
peptide is not particularly limited as long as it is
capable of being labeled with a metal. For example, a
leukocyte-binding compound can be used as the peptide

20 when used in the diagnosis of inflammation or the like.

[0014]

A compound represented by the following formula (1):

[Formula 1]

$Z-Y-Leu-Phe-(X)n-Lys(NH_2)m-\varepsilon(-(R)o-(T)1-U)$ (1)
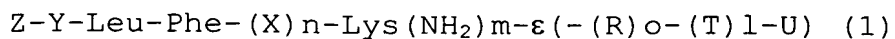
25 is preferably used as the peptide used in the present
invention. In this context, chemical formula (1)
consists of: Z-Y-Leu-Phe-, a formyl peptide receptor-
binding site of a leukocyte; -(R)o- a binding portion

11

that improves the rate of binding to monocytes and lymphocytes among all leukocytes; -U-, a structure capable of being labeled with a radioactive metal and paramagnetic metal; and -(X)n-, -Lys(NH$_2$)m-, and -(T)l-,

5   spacers that combine them.

In chemical formula (1), Z represents a protecting group for an amino group; Y represents Met or Nle; in (X)n, X represents a spacer consisting of one or more amino acids or a compound capable of being

10   organically synthesized, and n represents 1 or 0; in (NH$_2$)m, NH$_2$ represents an amide group serving as a protecting group for an α-carboxyl group of Lys, and m represents 1 or 0; and in ε(-(R)o-(T)l-U), R represents Ser or Thr bound via amide bond with an ε-amino group of

15   Lys, o represents 1 or 0, T represents a spacer consisting of one or more amino acids or a compound capable of being organically synthesized, l represents 1 or 0, and U represents a group capable of being labeled with a metal, wherein X and T may be identical

20   or different.

[0015]

In chemical formula (1), the group represented by U is not particularly limited as long as it is capable of being labeled with a metal. The group

25   is preferably a ligand consisting of several amino acids. More specific examples thereof include a tripeptide capable of being labeled with a metal, dipeptide-mercapto-acylate, a nitrogen-containing

cyclic compound having 8 to 20 carbon atoms, a
nitrogen-containing cyclic carboxylic acid compound
having 8 to 20 carbon atoms, a derivative of a
nitrogen-containing cyclic carboxylic acid compound

5   having 8 to 20 carbon atoms, and alkylene-amine-
carboxylic acid having 4 to 10 carbon atoms. To be
more specific, examples of the group that can be used
include a group capable of being labeled with a metal,
which is selected from a tripeptide capable of being

10   labeled with a metal such as -Cys-Gly-Asp, -Cys-Asp-
Asp, -Cys-Asp-Gly, -Cys-Gly-Glu, -Cys-Glu-Glu, -Cys-
Glu-Gly, -Cys-Gly-Asn, -Cys-Asn-Asn, -Cys-Asn-Gly, -
Cys-Gly-Gln, -Cys-Gln-Gln, -Cys-Gln-Gly, -Cys-Gly-Lys,
-Cys-Lys-Lys, -Cys-Lys-Gly, -Cys-Gly-Arg, -Cys-Arg-Arg,

15   and -Cys-Arg-Gly; dipeptide-mercapto-acylate such as -
Asp-Asp-mercaptoacetyl, -Gly-Asp-mercaptoacetyl, and -
Gly-Gly-mercaptoacetyl; a nitrogen-containing cyclic
compound having 8 to 20 carbon atoms such as 1,4,7,10-
tetraazacyclododecane (Cyclen), 1,4,8,11-

20   tetraazacyclotetradecane (Cyclam), 1,4,8,12-
tetraazacyclopentadecane, and 1,4,8,11-
tetraazacyclotetradecane-5,7-dione (Dioxocyclam); a
nitrogen-containing cyclic carboxylic acid compound
having 8 to 20 carbon atoms such as 1,4,8,11-

25   tetraazacyclotetradecane-1,4,8,11-tetraacetic acid
(TETA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-
tetraacetic acid (DOTA), 1,4,8,11-
tetraazacyclotetradecane-5,7-dione-N,N',N'',N'''-

tetraacetic acid, 1,4,7,10-tetraazacyclododecane-
butyric acid, and 1,4,8,10-tetraazacyclododecane-
butyric acid; a derivative of a nitrogen-containing
cyclic carboxylic acid compound having 8 to 20 carbon

5   atoms such as 1,4,7,10-tetraazacyclododecane-1-
aminoethylcarbamoylmethyl-4,7,10-tris(R,S)-methylacetic
acid (DO3MA), and 1,4,7,10-tetraazacyclododecane-
1,4,7,10-α,α',α'',α'''-tetramethylacetic acid (DOTMA);
and alkylene-amine-carboxylic acid having 4 to 10

10 carbon atoms such as ethylenediaminetetraacetic acid
(EDTA), diethylenetriaminepentaacetic acid (DTPA),
triethylenetetraminehexaacetic acid, and ethylene
glycol-(2-aminoethyl)-N,N,N',N'-tetraacetic acid
(EGTA).

15 [0016]

The specific compound represented by chemical
formula (1) is preferably a compound wherein in the
formula (1), Z represents a formyl group. Examples
thereof include N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-

20 ε(-Ser-Cys-Gly-Asn),
N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-Cys-Gly-
Asp),
N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-ε(-Ser-Cys-Asp-Asp),
N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-e(-Ser-D-Arg-Asp-

25 Cys-Asp-Asp),
N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-D-Arg-
diethylenetriaminepentaacetic acid (DTPA)),
N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-

diethylenetriaminepentaacetic acid (DTPA)),

N-formyl-Met-Leu-Phe-Lys-ε(-Asp-Asp-mercaptoacetyl),

N-formyl-Met-Leu-Phe-Lys-ε(-Gly-Asp-mercaptoacetyl),

N-formyl-Met-Leu-Phe-Lys-ε(-Gly-Gly-mercaptoacetyl),

5   N-formyl-Met-Leu-Phe-Lys-ε(-Asp-Gly-mercaptoacetyl),

N-formyl-Met-Leu-Phe-Lys-ε(diethylenetriaminepentaacetic

acid (DTPA)), and

N-formyl-Met-Leu-Phe-Lys-hydrazinonicotinic acid.

[0017]

10   More preferable examples thereof include N-

formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-Cys-Gly-Asn),

N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-Cys-Gly-

Asp),

N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-ε(-Ser-Cys-Asp-Asp),

15   N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-D-Arg-Asp-

Cys-Asp-Asp),

N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-D-Arg-

diethylenetriaminepentaacetic acid (DTPA)),

N-formyl-Met-Leu-Phe-Lys-ε(-Asp-Asp-mercaptoacetyl),

20   N-formyl-Met-Leu-Phe-Lys-ε(-Gly-Asp-mercaptoacetyl), and

N-formyl-Met-Leu-Phe-Lys-ε(-Gly-Gly-mercaptoacetyl).

When the compound represented by chemical

formula (1) is used in the present invention, a

compound in which the group capable of being labeled

25   with a metal is protected with an appropriate

protecting group can also be used.

[0018]

The above-described peptide capable of being

labeled with a metal such as the leukocyte-binding
compound can be synthesized by a method described
below.

(1)   A peptide consisting of amino acids can be
5 synthesized by a Boc method or Fmoc method or the like
using a generally used automatic peptide synthesizer
such as automatic peptide synthesizers manufactured by
Applied Biosystems. The synthesized complex, which is
bound with a resin carrier for a solid phase, is
10 simultaneously subjected to the removal of the
protecting group and the separation thereof from the
resin carrier and can be purified by high-performance
liquid chromatography (hereinafter, referred to as
HPLC) using a reverse-phase column or the like.
15 Alternatively, the peptide may be prepared by liquid-
phase peptide synthesis or may be collected from
animals and so on.

[0019]

(2)   A peptide which contains a non-amino acid
20 compound can be synthesized by the same method as above
in most cases. For example, a Lys residue or protected
derivative thereof is bonded to a resin carrier in a
solid phase. To the N terminus thereof, an amino acid
residue or protected derivative thereof or a compound
25 or protected derivative thereof with a function as a
spacer represented by X, a Phe residue or protected
derivative thereof, a Leu residue or protected
derivative thereof, and an amino acid or protected

derivative thereof represented by Y are sequentially

bonded thereto. Subsequently, the ε-amino group, a side

chain of Lys bound with the resin carrier for a solid

phase, is activated and bonded to Ser or Thr or

5 protected derivative thereof represented by R and then

to an amino acid or protected derivative thereof as a

spacer or a compound or protected derivative thereof

with a function as a spacer represented by T, and to a

compound or protected derivative thereof capable of

10 serving as the group capable of being labeled with a

metal represented by U. The synthesized product of

interest represented by the above-described formula (1)

can be separated from the resin carrier to synthesize

the peptide.

15   Other peptides capable of being labeled with

a metal can be synthesized easily by adopting the

above-described methods.

[0020]

      The amount of usage of the peptide capable of

20 being labeled with a metal in the medical composition

of the present invention can generally be in the range

preferable for diagnosis and treatment of interest.

This range is preferably a range acceptable in terms of

safety. When a peptide that acts on a receptor is

25 used, it is preferred that the peptide should be used

in an amount equal to or smaller than that of the

receptor of interest. To be more specific, the amount

of usage of the peptide is preferably in the

concentration range of 0.1 nM to 10 μM.

[0021]

The medical composition of the present invention is prepared by dissolving the above-described peptide capable of being labeled with a metal together with the basic organic compound in an aqueous solvent, specifically in a solvent of sterile water, if necessary supplemented with other organic solvents. A desired additive can further be added thereto, if necessary. Examples of the additive can include a surfactant, hydrophilic organic solvent, reductant, pH adjuster, and stabilizer.

[0022]

Examples of the surfactant can include, but not particularly limited to, nonionic surfactants such as polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan tristearate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan trioleate, polyethylene glycol 200, polyethylene glycol 300, polyethylene glycol 600, polyethylene glycol 1000, and polyethylene glycol 1540. Preferably, polyoxyethylene sorbitan monooleate is used. The amount of the surfactant added is preferably 0.01 to 1% by weight, more preferably 0.05 to 0.5% by weight, in the medical composition.

Examples of the hydrophilic organic solvent may include, but not particularly limited to, polar

organic solvents such as ethanol, propanol, butanol, acetonitrile, acetone, and methylethylketone. The amount of the hydrophilic organic solvent added is preferably 0.1 to 10% by weight, more preferably 0.2 to

5 1% by weight, in the medical composition.

The reductant is added for preventing the oxidation of the labeled metal. Examples of the reductant can include, but not particularly limited to, stannous chloride, ascorbic acid, and sodium

10 borohydride. The concentration of the reductant added is preferably in the range of 0.01 mM to 10 mM, more preferably 0.05 mM to 1 mM, in the medical composition.

Examples of the pH adjuster can include, but not particularly limited to, hydrochloric acid, acetic

15 acid, trifluoroacetic acid (hereinafter, referred to as "TFA"), citric acid, phosphoric acid, ascorbic acid, sodium hydroxide, and ammonia.

Examples of the stabilizer can include, but not particularly limited to, ascorbic acid, sodium

20 ascorbate, p-aminobenzoic acid, sodium p-aminobenzoate, gentisic acid, and sodium gentisate. The concentration of the stabilizer added is preferably in the range of 0.1 mM to 1 M, more preferably 5 mM to 500 mM, in the composition.

25 [0023]

The medical composition according to the present invention can be used in the form of an aqueous solution in which the peptide capable of being labeled

with a metal and the basic organic compound are

dissolved in the aqueous solvent, or can be prepared

into a freeze-dried form by freeze-drying the medical

composition in the form of an aqueous solution. The

5 freeze-dried medical composition is easily capable of

being used in kits or diagnostic drugs or

pharmaceutical drugs for therapeutic use in other forms

and can be used easily as a final material for an

injection. When the medical composition is freeze-

10 dried, the medical composition that is used in the form

of an aqueous solution in which the peptide capable of

being labeled with a metal and the basic organic

compound are dissolved in the aqueous solvent can be

freeze-dried by a usual method.

15 The medical composition of the present

invention in the form of an aqueous solution or in the

freeze-dried form can be applied together with a

labeling reagent containing a metal and so on to a

diagnostic drug or a pharmaceutical drug for

20 therapeutic use.

[0024]

The peptide capable of being labeled with a

metal in the medical composition of the present

invention can be labeled efficiently with a metal. A

25 medical preparation prepared by labeling the medical

composition with a metal can be accumulated in

inflammation sites with immune response by the action

of the peptide and can image the above-described sites

by the ·labeled metal, for example when a peptide such
as a leukocyte-binding compound that can be utilized in
the imaging of inflammation sites is used as the
peptide capable of being labeled with a metal. To be
5 more specific, the use of the medical preparation
according to the present invention as a radioactive
diagnostic agent and an MRI contrast agent allows for
the imaging of the inflammation sites. The preparation
of the present invention can be used not only in
10 imaging but also as a radioactive therapeutic agent by
selecting a proper radioactive metal.

[0025]

When the preparation is used as a radioactive
diagnostic agent, it is preferred that the metal-
15 labeled peptide in the medical composition of the
present invention should be a radioactive metal-labeled
peptide that is labeled with a radioactive metal for
SPECT such as Tc-99m, In-111, Ga-67, Sn-117m, Sm-153,
and Re-186, or a radioactive metal for PET such as Cu-
20 64 and Ga-68. When the preparation is used as an MRI
contrast agent, it is preferred that the metal-labeled
peptide should be a paramagnetic metal-labeled peptide
in which a paramagnetic metal such as Cu, Fe, Mn, Gd,
and Dy is coordinated to the above-described peptide.
25 When the preparation is used as a radioactive
therapeutic agent, it is preferred that the metal-
labeled peptide should be a radioactive metal-labeled
peptide in which the above-described peptide is labeled

with a radioactive metal such as Y-90, Re-186, and Re-188.

[0026]

A variety of methods can be used for labeling
the above-described peptide with a metal, for example a
radioactive metal or paramagnetic metal. For example,
when the peptide is labeled with Tc-99m, Re-186, or Re-188, a labeled compound can be prepared by a routine
method in which a stable amount of a reductant
acceptable as a pharmaceutical additive such as
stannous chloride is added to the medical composition
of the present invention and then mixed with a sodium
pertechnetate solution or sodium perrhenate solution.
When the peptide is labeled with Cu, Cu-64, Fe, Mn, Gd,
In-111, Sn-117m, Sm-153, or Dy, a labeled compound can
be prepared by mixing the medical composition according
to the present invention with a weakly acid, water-soluble solution containing Cu, Cu-64, Fe, Mn, Gd, In-111, Sn-117m, Sm-153, or Dy ions. In this case, it is
preferred that the final pH should be adjusted to 8 or
higher. When the peptide is labeled with Ga-67, Ga-68,
or Y-90, the labeling may be performed by mixing the
medical composition according to the present invention
with a weakly acid or alkaline, water-soluble solution
containing Ga-67, Ga-68, or Y-90 ions. In this case,
it is preferred that the final pH should be adjusted to
8 or higher.

The peptide may be labeled with a metal at

room temperature for 5 minutes to 24 hours, preferably

5 to 60 minutes, more preferably 10 to 30 minutes, with

stirring, shaking, or the like.

[0027]

5   The medical preparation comprising the

radioactive metal- or paramagnetic metal-labeled

peptide is further supplemented with a

pharmacologically acceptable additive, if necessary,

and can be prepared into a preferable radioactive

10   diagnostic agent or radioactive therapeutic agent.

Examples of the additive include: a stabilizer such as

pharmacologically acceptable ascorbic acid, sodium

ascorbate, p-aminobenzoic acid, sodium p-aminobenzoate,

gentisic acid, and sodium gentisate; a pH adjuster such

15   as aqueous buffer solutions; an excipient such as D-

mannitol; and citric acid, tartaric acid, malonic acid,

sodium gluconate, and sodium glucoheptonate, which are

useful for improving radiochemical purities.

[0028]

20   When the medical preparation of the present

invention comprising the radioactive metal- or

paramagnetic metal-labeled peptide is used as a

radioactive diagnostic agent, radioactive therapeutic

agent, or MRI contrast agent, the preparation can be

25   administered by generally used parenteral means such as

intravenous administration. The dose thereof is

determined in consideration of conditions such as the

body weight and age of a patient, an appropriate

radiographic imaging apparatus or MRI measuring apparatus, and symptoms of target disease.

For example, when a human is targeted, the dose of a diagnostic agent using the Tc-99m-labeled peptide is in the range of 37 MBq to 1110 MBq, preferably 185 MBq to 1110 MBq. The dose of a therapeutic agent using the Re-186- or Re-188-labeled peptide is in the range of 37 MBq to 18500 MBq, preferably 370 MBq to 7400 MBq. The dose of a therapeutic agent using the Y-90-labeled peptide is in the range of 37 MBq to 3700 MBq, preferably 37 MBq to 1110 MBq. The doses of agents using the labeled peptides that are labeled with other radioactive metals are almost the same as above. The dose of a therapeutic agent using the labeled peptide that is labeled with a paramagnetic metal such as Gd, Fe, Mn, Cu, and Dy is appropriately selected according to the sensitivity of an MRI imaging apparatus, a target tissue, a particular administration style, and the intended effect of usage.

For example, when the preparation using Gd as the metal is intravenously administered to a human, the dose thereof that is preferably used is usually 0.01 to 0.3 mmol/kg of body weight.

[0029]

Hereinafter, the present invention will be described more fully with reference to Examples of the present invention. However, the present invention is

not limited to these Examples by any means.

A method for measuring substances obtained in Examples, reagents used, etc will be shown below.

(1)   Gamma counter: distribution in blood was

5   measured with an Auto Well Gamma Counter (manufactured by Aloka). In in-vivo distribution studies, measurement was performed with NaI Single-Channel Analyzer (manufactured by Ohyo Koken Kogyo).

(2)   Gamma camera: measurement was performed with

10 GMS-550U (manufactured by Toshiba medical) or Millennium MG (manufactured by GE Yokogawa Medical Systems).

(3)   Reverse-phase HPLC: a reverse-phase column Cosmosil $5C_{18}$-AR-300 (manufactured by Nacalai Tesque,

15 4.6×150 mm) was used.

(4)   All peptide compounds were produced by solid-phase synthesis.

(5)   Those eluted in a physiological saline by use of a $^{99m}TcO_4-$:^{99}Mo/^{99m}Tc generator (trade name: Meditech,

20 manufactured by Nihon Medi-Physics) were used.

(6)   All reagents used were special grade or higher grade products.

(7)   All experimental animals were raised for 1 week under conditions of a cycle of 12-hour light and

25 12-hour dark prior to experiments. During this period, they freely consumed foods and water.

[0030]

(8)   Peptides used in the present Examples were

peptides described below.

Peptide 1: formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-D-Arg-Asp-Cys-Asp-Asp)

Peptide 2: formyl-Nle-Leu-Phe-Nle-Tyr-Lys(NH$_2$)-ε(-Ser-D-Arg-DTPA*)

Peptide 3: formyl-Met-Leu-Phe-Lys-ε(-Gly-Asp-Ac-S-Bzl)

*: DTPA: diethylenetriaminepentaacetic acid

[0031]

(9)   Synthesis of Peptide 1

Peptide 1 was produced by solid-phase synthesis and used in Examples described below.

A peptide synthesizer (model: 430A) manufactured by Applied Biosystems was used to synthesize a peptide by a Boc method using an MBHA resin (p-Methoxy-Benzhydrylamine Resin hydrochloride, 1% Divinylbenzene-polystyrene copolymer) under conditions of 0.5 mM scales. In this procedure, the C terminus and the side chain of the Lys residue were protected with Fmoc groups. The peptide chain was elongated, and the N-terminal amino group was formylated. The Fmoc group of the side chain of the Lys residue was cleaved with 20% piperidine/DMF, and the peptide chain was elongated in the direction of the side chain. The peptide was separated therefrom through its reaction at -2°C to -5°C for 1.0 hour in anhydrous hydrogen fluoride:p-cresol (80:20).

Main peaks of the synthesized peptide were fractionated by HPLC (Column: YMC-Pack ODS-A SH-365-5

(trade name, manufactured by YMC, 30×250 mm), Column
temperature: room temperature, Elution rate: 20 mL/min,
Detector: ultraviolet and visible spectrophotometer
(absorption wavelength: 220 nm), Eluant A: 0.1%

5   TFA/purified water, Eluant B: 0.1% TFA/acetonitrile
(A/B (80%/20%) → A/B (30%/70%), 90 min)), followed by
freeze drying. The obtained peptide was analyzed by
reverse-phase HPLC (Column: Zorbax 300SB-C18 (trade
name, manufactured by Yokogawa Analytical Systems,

10   4.6×150 mm, Column temperature: 50°C, flow rate: 1.0
mL/min, Detector: ultraviolet and visible
spectrophotometer (absorption wavelength: 220 nm),
Eluant A: 0.1% TFA/purified water, Eluant B: 0.1%
TFA/acetonitrile (A/B (80%/20%) → A/B (30%/70%), 25

15   min)). As a result, the purity of the obtained peptide
was 93.2% (area percentage).

The peptide could also be synthesized by
using a preloaded resin instead of the MBHA resin.
[0032]

20   The above-described purified peptide was
decomposed into each amino acid through its hydrolysis
in 6 M hydrochloric acid at 110°C for 22 hours. Amino
acid composition was determined with L-8800-model
Hitachi High-Speed Amino Acid Analyzer (trade name,

25   manufactured by Hitachi High-Technologies) under
conditions described below to confirm that the amino
acids had the same amino acid composition as that of
Peptide 1. Moreover, mass spectrometry (hereinafter,

ESI-MS) was conducted to confirm that the results of the amino acids were identical to the theoretical value of Peptide 1.

Analytical conditions for amino acid composition

Model: L-8800-model Hitachi High-Speed Amino Acid Analyzer

Chromatography conditions

Column size: 4.6 mm I.D.×60.0 mm

Stationary phase: Hitachi Custom Ion-Exchange Resin #2622

Mobile phase: Step-wise elution with sodium citrate buffer solutions (4 types, and for regeneration of analytical column, 0.2 N NaOH test solution)

     (1) L-8500-PH1: 0.16 N, pH 3.3

     (2) L-8500-PH2: 0.2 N, pH 3.2

     (3) L-8500-PH3: 0.2 N, pH 4.0

     (4) L-8500-PH4: 1.2 N, pH 4.9

Flow rate: 0.40 mL/min

Column temperature: 57°C

Detection

Reagent: (1) ninhydrin test solution, (2) buffer solution for ninhydrin (Ninhydrin Reagent L-8500 Set)

Flow rate of reaction reagent: 0.35 mL/min

Reaction apparatus: electron heating reaction column,

Temperature set: 135°C

Detection wavelength: 570 nm and 440 nm

Standard amino acid

Reagent: Ajinomoto Amino Acid Calibration Mixture

Concentration: 50 nmol/mL each (however, 100 nmol/mL for Pro)

Diluent: 0.2 M Na Citrate Buffer (pH 2.2)

Injected amount: 40 µL (2 nmol each of amino acids and

5 4 nmol of Pro)

Calculation method for quantification: one-point absolute calibration curve method on the basis of area values

The analysis value (the number of amino acids

10 per molecule) of amino acid composition of the obtained Peptide 1 and a result of mass spectrometry thereof are shown below. Numerals in parentheses represent the theoretical values of amino acid composition of the peptide of interest.

15 [0033]

Peptide 1=Asp: (3) 3.19, Ser: (1) 0.97, Tyr: (1) 0.97, Phe: (1) 0.98, Lys: (1) 1.00, NH_3 (1) 1.20, Leu (1)+ Nle (2) 2.80, Arg: (1) 1.06, Cys: (1) 0.92

The analysis value of ESI-MS of the obtained

20 Peptide 1 is shown below. A numeral in a parenthesis represents the theoretical value of a molecular weight of the peptide of interest.

ESI-MS: MW=514.4 (1514.7)

Example 1

25 [0034]

Solubility of peptide by arginine

(1) Procedures

Peptide 1, Peptide 2, and Peptide 3 (100 µg

each) were separately added to 410 mM arginine solutions (pH 11) to adjust each total amount to 700 μL. Molarities in these solutions are 94 μM (Peptide 1), 99 μM (Peptide 2), and 161 μM (Peptide 3). The presence or absence of white turbidity was confirmed by external observation. Then, 200 μL each of the sample solutions was filtered with a filter Millex (registered trademark)-GV (trade name, manufactured by Nihon Millipore) with a pore size of 0.22 μm. The sample solutions before filtration and the sample solutions after filtration were respectively analyzed by reverse-phase HPLC. Peak area percentages were determined from the peak area values of the peptides before and after filtration and used as elution rates. HPLC analytical conditions are shown below.

For a comparative example, Peptide 1, Peptide 2, and Peptide 3 were separately dissolved in each of water and dimethylsulfoxide (DMSO) to prepare samples having a peptide concentration of 94 μM, 99 μM, or 161 μM, which were studied in the same way as above.

HPLC conditions

Column: Cosmosil $5C_{18}$-AR-300 (Manufactured by Nacalai Tesque, 4.6×150 mm)

Elution rate: 1 mL/min

Detection: ultraviolet and visible spectrophotometer (detection wavelength: 220 nm)

HPLC system: Alliance (manufactured by Waters, Japan)

Eluant A: 0.1% trifluoroacetic acid
(hereinafter, TFA)/purified water

Eluant B: 0.1% TFA/acetonitrile

Concentration gradient: 0 min (Eluant B 20%)

5 → 25 min (Eluant B 70%)

[0035]

(2) Result

The obtained result is shown in Table 1 and
Fig. 1. As a result of external observation after each
10 peptide dissolution, white turbidity was observed in 3
types of peptides dissolved in water, whereas all 3
types of peptides exhibited colorless, transparent
appearances in their aqueous solutions of arginine.
Similar appearances were exhibited in the sample
15 solutions of DMSO. For elution rates obtained by
reverse-phase HPLC, all of the respective sample
solutions of arginine and DMSO exhibited approximately
100% elution rates. These results demonstrated that
arginine enhances peptide solubility, as with DMSO.
20 [0036]

[Table ·1]

Result of external observation and elution rate of each sample solution

Solvent / Peptide	Appearance			Elution rate	
	Water	Arginine	DMSO	Arginine	DMSO
Peptide 1	Whitish	Colorless, transparent	Colorless, transparent	100.8%	98.6%
Peptide 2	Whitish	Colorless, transparent	Colorless, transparent	102.8%	100.9%
Peptide 3	Whitish	Colorless, transparent	Colorless, transparent	101.3%	108.5%

Example 2

[0037]

Solubility of peptide 1 by arginine, lysine, and histidine at each pH

(1) Procedures

Arginine, lysine, and histidine were separately dissolved in water to prepare 410 mM arginine solution, 489 mM lysine solution, and 90 mM histidine solution. An aqueous solution of sodium hydroxide and hydrochloric acid were added to these solutions to adjust the pH of the aqueous solutions of lysine and histidine to 8, 9, 10, and 11, and the pH of the aqueous solution of arginine to 8, 8.5, 9, 9.5, 10, and 11. Subsequently, Peptide 1 was added to these aqueous solutions to prepare 94 μM Peptide 1 solutions. The presence or absence of white turbidity was confirmed by external observation. Then, 200 μL each of the sample solutions was filtered with a filter

Millex (registered trademark)-GV (trade name,

manufactured by Nihon Millipore) with a pore size of

0.22 μm. The sample solutions before filtration and

the sample solutions after filtration were respectively

5   analyzed by reverse-phase HPLC. Peak area percentages

were determined from the peak area values of Peptide 1

before and after filtration and used as elution rates.

HPLC analytical conditions were the same as the

conditions described in Example 1.

10   [0038]

(2) Result

The obtained result is shown in Table 2.

Evident white turbidity was observed in the aqueous

solution of arginine at pH 8. Weak white turbidity was

15   observed in the respective aqueous solutions of

arginine, lysine, and histidine at pH 8.5 or less,

whereas elution rates slightly more than 20% were

observed in the aqueous solutions of lysine and

histidine even at pH 8. These results demonstrated

20   that lysine and histidine have peptide-dissolving

effect even at pH 8. The aqueous solutions of

arginine, lysine, and histidine became colorless,

transparent solutions at pH 9 or more, and the elution

rates of the peptide were 85% or more. Thus, these

25   basic amino acids were shown to be capable of producing

peptide solubility at pH 8 or more.

[0039] ·

[Table 2]

Solubility of peptide 1 by arginine, lysine, and

hystidine at each pH

	pH 8	pH 8.5	pH 9	pH 9.5	pH 10	pH 11
Arginine	Not measured (*)	43.8%	93.8%	95.4%	98.8%	100.8%
Lysine	21.0%	–	89.0%	–	83.6%	85.9%
Histidine	26.8%	–	85.9%	–	90.7%	96.7%

* Not measured by HPLC due to evident white turbidity
  confirmed in external observation

Example 3

[0040]

Solubility of Peptide 1 by imidazole at each

pH

5 (1) Procedures

Imidazole was dissolved in water to prepare 1

mM imidazole solution. pH was adjusted to 8, 8.5, and

9 by adding an appropriate amount of hydrochloric acid

to this solution. Subsequently, Peptide 1 was added to

10 these imidazole solutions to prepare 94 µM Peptide 1

solutions. The presence or absence of white turbidity

was confirmed by external observation. Then, 200 µL

each of the sample solutions was filtered with a filter

Millex (registered trademark)-GV (trade name,

15 manufactured by Nihon Millipore) with a pore size of

0.22 µm. The sample solutions before filtration and

the sample solutions after filtration were respectively

analyzed by reverse-phase HPLC. Peak area percentages

were determined from the peak area values of Peptide 1 before and after filtration and used as elution rates. HPLC analytical conditions were the same as the conditions described in Example 1.

5 [0041]

(2) Result

The obtained result is shown in Table 3. All the solutions with pH 8, 8.5, and 9 were colorless and transparent and exhibited 80% or more elution rates at

10 pH 8.5 and 9 or more. These results demonstrated that imidazole imparts high solubility to Peptide 1.

[0042]

[Table 3]

Result of external observation and elution rate of

15 Peptide 1 by imidazole at each pH

	Appearance	Elution rate
pH 8	colorless, transparent	64.9%
pH 8.5	colorless, transparent	80.8%
pH 9	colorless, transparent	99.2%

Example 4

[0043]

Solubility of Peptide 1 by a variety of additives

20 (1) Procedures

Disodium hydrogen phosphate, sodium dihydrogen citrate, sodium ascorbate, and imidazole were separately dissolved in water to prepare their respective aqueous solutions with pH 9 at

25 concentrations described in Table 4. pH adjustment was

performed with hydrochloric acid and an aqueous
solution of sodium hydroxide. The above-described
aqueous solutions were separately added to the weighed
Peptide 1 to prepare 94 μM Peptide 1 solutions. The

5   presence or absence of white turbidity was confirmed by
external observation. Similarly, the presence or
absence of white turbidity was confirmed for the
aqueous solutions of Peptide 1 dissolved in the aqueous
solutions of arginine, lysine, and histidine, which

10   were obtained in Example 2. These Peptide 1 solutions
were compared with the Peptide 1 sample solutions using
the respective aqueous solutions of disodium hydrogen
phosphate, sodium dihydrogen citrate, and sodium
ascorbate used as comparative controls. Subsequently,

15   200 μL each of the sample solutions was filtered with a
filter Millex (registered trademark)-GV (trade name,
manufactured by Nihon Millipore) with a pore size of
0.22 μm. The sample solutions before filtration and
the sample solutions after filtration were respectively

20   analyzed by reverse-phase HPLC. Peak area percentages
were determined from the peak area values of Peptide 1
before and after filtration and used as elution rates.
pH was measured after HPLC measurement and used as
actually measured pH values. HPLC analytical

25   conditions were the same as the conditions described in
Example 1.

[0044] ·

[Table 4]

Concentration in sample solution of each additive

Solvent	Additive	Morality	Weight concentration	pH
Water	Arginine	410 mM	71.4 mg/mL	9
	Histidine	90 mM	14 mg/mL	9
	Lysine	489 mM	50 mg/mL	9
	Disodium hydrogen phosphate	100 mM	14 mg/mL	9
	Sodium ascorbate	401 mM	71.4 mg/mL	9
	Sodium dihydrogen citrate	243 mM	71.4 mg/mL	9
	Imidazole	1 mM	0.07 mg/mL	9

[0045]

(2) Result

The obtained result is shown in Table 5 and
Fig. 2. As a result of dissolution of Peptide 1, the
aqueous solutions of 3 types of basic amino acids,
arginine, lysine, and histidine, at pH 9 exhibited
colorless, transparent appearances. The Peptide 1
sample solution using the aqueous solution of imidazole
also exhibited a colorless, transparent appearance.

On the other hand, the respective aqueous
solutions of sodium ascorbate and sodium dihydrogen
citrate used as comparative controls exhibited white
turbidity after the addition of Peptide 1. Although
the aqueous solution of disodium hydrogen phosphate
exhibited a colorless, transparent appearance after the
addition of Peptide 1, its elution rate calculated by

HPLC analysis exhibited a value of 53.8% and was low as compared with those of 3 types of basic amino acids and imidazole. These results demonstrated that these 3 types of basic amino acids and imidazole have not only solubility-promoting action by pH but also the action of enhancing the solubility of Peptide 1.

[0046]

[Table 5]

Appearances, elution rate, and actually measured pH values of Peptide 1 solutions using a variety of aqueous solutions

Solvent	Additive	pH	Elution rate	pH after preparation	Appearance
Water	Arginine	9	93.8%	8.97	Colorless, transparent
	Histidine	9	85.9%	9.00	Colorless, transparent
	Lysine	9	89.0%	8.99	Colorless, transparent
	Disodium hydrogen phosphate	9	53.8%	8.85	Colorless, transparent
	Sodium ascorbate	9	Not measured (*)	9.10	Whitish
	Sodium dihydrogen citrate	9	Not measured (*)	9.05	Whitish
	Imidazole	9	99.2%	9.10	Colorless, transparent

* Not measured by HPLC due to evident white turbidity
  confirmed in external observation

Example 5

[0047]

Tc99m labeling-promoting effect by arginine

(1) Procedures

Peptide 1 was dissolved in the aqueous solution of arginine with pH 10 obtained in Example 2 to prepare 9.4 µM peptide/arginine solution. 25 µL of a solution of 10 mL of 0.01 M hydrochloric acid

5   supplemented with 5 mg of stannous chloride solution was added to 700 µL of the peptide/arginine solution, and 0.6 to 1.0 GBq of Tc-99m-sodium pertechnetate (hereinafter, 99mTcO$_4$$^-$) solution was quickly added thereto to adjust the total amount to 1 mL. After the

10   labeling procedure, the peptide concentration was diluted to 6.6 µM. After shaking for several seconds, the solution was reacted at room temperature. A portion thereof was taken on 10 minutes and 90 minutes after the labeling procedure to determine each Tc-99m

15   labeling rate by HPLC and TLC. The HPLC and TLC analyses were performed under conditions described below.

For comparative controls, Peptide 1 was dissolved in dimethylsulfoxide (DMSO) and then diluted

20   10-fold with 100 mM phosphate buffer solution (hereinafter, PB) with pH 10 to prepare a peptide/DMSO/PB solution with the final peptide concentration of 9.4 µM. Alternatively, Peptide 1 was dissolved in PB to prepare a peptide/PB solution with

25   the final peptide concentration of 9.4 µM. These solutions were labeled in the same way as in the peptide/arginine solution to determine each Tc-99m labeling rate by HPLC and TLC. After the labeling

procedure, the peptide concentration was diluted to 6.6 μM.

HPLC conditions

Column: Cosmosil $5C_{18}$-AR-300 (manufactured by Nacalai Tesque, 4.6×150 mm)

Elution rate: 1 mL/min

Detection: ultraviolet and visible spectrophotometer (detection wavelength: 220 nm)

Radioactivity detector: STEFFI NaI scintillator (manufactured by Raytest)

Eluant A: 0.1% TFA/purified water

Eluant B: 0.1% TFA/acetonitrile

Concentration gradient: 0 min (Eluant B 20%) → 25 min (Eluant B 70%)

TLC conditions

Plate: Silica Gel 60F254 (manufactured by Merck)

Developing solvent: 28% ammonia water/acetonitrile=1/2

Radioactivity detector: Gita NaI scintillator (manufactured by Raytest)

[0048]

(2) Result

Radiochemical purities calculated from the resulting peak areas are shown in Table 6. The peptide solution using 410 mM arginine solution exhibited 90% or more radiochemical purity on 10 minutes after labeling and 95% or more radiochemical purity up to 90

minutes· after labeling in HPLC analysis. Furthermore,
it also exhibited 85% or more radiochemical purity on
10 minutes and 90 minutes after labeling in TLC
analysis and was shown to have a high Tc99m labeling

5   rate and high labeling stability without the need of
heating.

On the other hand, radiochemical purities
exhibited by the peptide/DMSO/PB solution and the
peptide/PB solution used as comparative controls were

10   72.0% on 10 minutes after labeling and 75.4% on 90
minutes after labeling in HPLC analysis for the
peptide/DMSO/PB solution, 45.7% on 10 minutes after
labeling and 41.9% on 90 minutes after labeling in TLC
analysis for the peptide/DMSO/PB solution, 55.3% on 10

15   minutes after labeling in HPLC analysis for the
peptide/PB solution, and 28.0% on 10 minutes after
labeling and 33.6% on 90 minutes after labeling in TLC
analysis for the peptide/PB solution.

These results demonstrated that the

20   peptide/arginine solution has a radiochemical purity
higher than those of the comparative controls at both
points in time. Accordingly, it was shown that the use
of a dissolution method involving arginine addition can
achieve a high labeling rate in a nonheated manner and

25   improves the stability of the prepared labeled
material.

[0049] ·

[Table 6]

Radiochemical purity (%) in each solvent (pH10) and temporal shifts thereof

	Arginine		DMSO/phosphate buffer solution		Phosphate buffer solution	
	HPLC	TLC	HPLC	TLC	HPLC	TLC
10 minutes	92.5	87.2	72.0	45.7	55.3	28.0
90 minutes	96.5	88.6	75.4	41.9	−	33.6

Example 6

[0050]

Confirmation of Tc99m labeling at varying arginine concentrations and stability thereof

(1) Procedures

12.5, 25, and 50 mg of arginine was weighed, then dissolved in water, and adjusted with hydrochloric acid to pH 10 to adjust each total amount to 700 µL. Molarities in these solutions are 102.5 mM, 205 mM, and 410 mM, respectively. Peptide 1 was dissolved in these aqueous solutions of arginine to prepare respective 9.4 µM peptide/arginine solutions. 25 µL of a solution of 10 mL of 0.01 M hydrochloric acid supplemented with 5 mg of stannous chloride solution was added to each of the peptide/arginine solutions, and 0.6 to 1.0 GBq of 99mTcO$_4^-$ solution was quickly added thereto to adjust each total amount to 1 mL. After shaking for several seconds, the solutions were reacted at room temperature. A portion thereof was taken on 30 minutes, 90 minutes, 180 minutes, 360 minutes, 24

hours, ·and 30 hours after the labeling procedure to determine each radiochemical purity by TLC analysis under the conditions described in Example 5.

[0051]

5 (2) Result

Radiochemical purities calculated from the peak areas are shown in Table 7. The peptide/arginine solutions exhibited 80% or more radiochemical purities in all the results up to 30 hours after labeling and

10 further exhibited 85% or more radiochemical purities at arginine concentrations of 205 mM or less up to 30 hours after labeling. These results demonstrated that a dissolution method using arginine is capable of providing a Tc99m-labeled peptide exhibiting a high

15 Tc99m labeling rate and high labeling stability without the need of heating in a manner independent of arginine concentrations.

[0052]

[Table 7]

20 Radio chemical purity (%) at varying arginine concentrations and temporal shifts thereof

Elapsed time	Radiochemical purity (%) at varying arginine concentrations		
	102.5 mM	205 mM	410 mM
30 minutes	93.3%	92.0%	91.6%
90 minutes	92.6%	90.8%	89.5%
180 minutes	92.6%	89.7%	87.1%
360 minutes	91.2%	89.7%	85.7%
24 hours	88.9%	88.3%	86.5%
30 hours	89.8%	87.3%	84.9%

Example 7

[0053]

Imaging of rabbit model of infectious disease injected
of Tc99m-Peptide 1 solution prepared by arginine

5 addition in rabbit model of infectious disease

(1) Procedures

Approximately 10^8 viable cells of
Staphylococcus aureus were suspended in 1 mL of
physiological saline. A 100-µL aliquot thereof was

10 intramuscularly administered to the right thigh of each
rabbit (New Zealand White, male, body weight of
approximately 2 kg). After a lapse of 24 hours, the
rabbit model in which inflammation was evidently
observed was treated with pentobarbital anesthesia, and

15 37 to 74 MBq of the Tc99m-labeled Peptide 1
(arginine/nonheated) obtained in Example 5 was
intravenously administered to the ear to take images
with a gamma camera on 5 minutes, 1 hour, 2 hours, 3
hours, 4 hours, and 5 hours after administration. For

20 a comparative control, Tc99m-labeled Peptide 1
(DMF/heated) prepared with peptide/dimethylformamide
(DMF)/water and then heated was administered and
studied in the same way. Procedures for preparing the
comparative control are as follows: 1.1 to 3.0 GBq of

25 $^{99m}TcO_4^-$ solution was added into a vial containing a
mixture solution of 300 µL of 134 mM glucoheptonic acid
and 50 µL of 2.6 mM stannous chloride solution to
adjust each total amount to 1.35 mL. The solutions

were stirred by occasional inversion and reacted at room temperature for 30 minutes. A portion thereof was taken to confirm by cellulose acetate membrane electrophoresis that the Tc-99m labeling rate of Tc-

5 99m-glucoheptonic acid was 95% or more. Next, Peptide 1 was dissolved in dimethylformamide (DMF) to prepare 943 μM Peptide 1 solution, which was in turn diluted 10-fold with water to prepare a 94 μM Peptide 1 solution. The Tc-99m-glucoheptonic acid solutions (300

10 μL each) were separately added to 700 μL of this solution, then mixed by stirring, and heated to 100°C to 120°C to react the solutions for 10 minutes. After labeling, a portion thereof was taken to determine each Tc-99m labeling rate by HPLC. HPLC conditions are as

15 follows:

HPLC conditions

Column: Puresil 5 μm C18 (manufactured by Millipore, 4.6×150 mm)

Elution rate: 1 mL/min

20 Detection: ultraviolet and visible spectrophotometer (detection wavelength: 220 nm)

Radioactivity detector: STEFFI NaI scintillator (manufactured by Raytest)

Eluant A: 0.1% TFA/purified water

25 Eluant B: 0.1% TFA/acetonitrile

Concentration gradient: 0 min (Eluant B 20%) → 25 min (Eluant B 70%)

[0054]

(2.) Result

Representative diagrams of the obtained
results are shown in Figs. 3 and 4. A result of
setting a region of interest on the image to determine
5   the proportion (% ID/K pixel) of a count per 1000
pixels of each region of interest with respect to the
whole body count is shown in Table 8 and Fig. 5. As a
result, infection sites could be visualized clearly in
both the Tc99m-Peptide 1 (DMF/heated) of a conventional
10   technique and the Tc99m-Peptide 1 (arginine/nonheated)
of the present invention, and their visualized patterns
were common to each other. The accumulation to
inflammation of the formulation of the present
invention, that is, the solution prepared by arginine
15   addition under nonheated conditions exceeded that of
the conventional method, that is, the solution prepared
by DMF addition and heated to 100°C or higher at all the
points in time, and was increased from 1.59±0.32% ID/K
pixel on 1 hour after administration to 2.62±0.55% ID/K
20   pixel on 5 hours after administration. These results
demonstrated that the method of the present invention
in which a leukocyte-binding compound was dissolved in
arginine and labeled with Tc99m in a nonheated manner
can provide labeled materials having higher
25   accumulation properties to inflammation than those of
labeled materials obtained by conventional techniques.

[0055] ·

[Table 8]

Accumulation (% ID/k pixel) of Peptides 1 labeled with Tc99m to inflammation in rabit model of infectious disease (N=3, mean ± standard deviation)

Labeling method.	Elapsed time after administration					
	5 minutes	1 hour	2 hours	3 hours	4 hours	5 hours
Arginine/ nonheated	1.74± 0.22	1.59± 0.32	1.77± 0.25	2.01± 0.29	2.32± 0.56	2.62± 0.55
DMF/heated	0.95± 0.24	0.91± 0.14	1.09± 0.22	1.52± 0.27	1.76± 0.39	1.84± 0.27

INDUSTRIAL APPLICABILITY

[0056]

5   The use of a medical composition according to the present invention improves the solubility of a peptide capable of being labeled with a metal, which is insoluble or poorly soluble in an aqueous solvent and allows for the metal-labeling of the above-described
10 peptide without heating. The use of a metal-labeling method according to the present invention allows for the labeling of the above-described peptide with a metal under nonheated conditions. Furthermore, a preparation obtained by labeling, with a metal, the
15 above-described peptide in the medical composition according to the present invention has the advantage of improving the accumulation rate of the peptide to inflammation as compared with that of a composition prepared by a conventional method, for example when the
20 peptide that can be used in the imaging of inflammation

is' used' therein. For example when a leukocyte-binding compound is used as the peptide, the present invention is capable of providing a medical composition and a medical preparation and a labeling method thereof being

5   useful in PET diagnostic imaging, SPECT diagnostic imaging, and MRI diagnostic imaging that conduct the imaging of active leukocyte infiltration sites with the immune responses of individuals, or in radiation therapy.